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# Extended Literature Review Concerning NOAEL and LOAEL Values for Perchlorate

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## ES EXECUTIVE SUMMARY

The Perchlorate Study Group (PSG) conducted an initial literature review of NOAEL and LOAEL values for perchlorate in May 1994. The following month, after meeting with USEPA's Environmental Criteria and Assessments Office, the PSG decided to conduct a more thorough review of the available toxicological literature concerning perchlorate, which is this document. Herein we attempt to codify the available epidemiologic and experimental data into a format which facilitates establishment of a reference dose. These toxicological data for perchlorate include interspecies comparisons, sexual differences, genotoxic, developmental and reproductive data, as well as a discussion of potential target organ sites other than the thyroid gland.

As summarized in Section 6, there is a surprisingly good concordance of available toxicological data in several human studies and in experimental animals. The highest NOAEL and lowest LOAEL data in humans, rats and other animals are within an order of magnitude, with 12 mg/kg/day representing a NOAEL/LOAEL interface in healthy human volunteers. Males and females have equivalent sensitivities to perchlorate toxicity. Only the thyroid gland is adversely affected by perchlorate doses which are below gram/day levels in humans. The NOAEL for reproductive and developmental effects, including fetotoxicity, is also well above the threshold for perturbation of the thyroid-pituitary axis.

It is recommended that the recently determined NOAEL/LOAEL value of 12 mg/kg/day perchlorate be adopted as the basis for a reference dose with application of three-fold safety factors for subchronic-to-chronic extrapolation and for protection of potentially sensitive populations.

# 1.0 INTRODUCTION

## 1.1 *Overview of the Perchlorate Anion*

Perchlorate, $ClO_4^-$, is an anion which forms salts with most cations. Monovalent cation salts of sodium ($NaClO_4$), potassium ($KClO_4$) and ammonium ($NH_4ClO_4$) perchlorate have found wide use as rocket propellants, ignitable sources and, medicinally, for control of hyperthyroidism. Perchlorate is still used today for the control of hyperthyroidism in Germany. Since perchlorate salts all dissociate completely when dissolved in water or aqueous tissues, their toxicities are equivalent (although doses must be adjusted slightly to account for molecular weight differences of the cations). As will be annotated further below, the sole toxicologic mechanism of perchlorate in the 1-10 mg/kg/day dose range in a variety of mammalian systems is to block iodine uptake by the thyroid gland.

## 1.2 *Meeting with ECAO*

The Perchlorate Study Group (PSG), a group of seven companies which manufacture or use perchlorates, met with the USEPA's Environmental Criteria and Assessments Office (ECAO) in Cincinnati, OH, on 30 June 1994. Previously, in December 1992, ECAO had issued a preliminary paper in which it had derived a provisional reference dose (RfD) for perchlorate (ECAO, 1992). In May 1994 the PSG had drafted its own review of pertinent perchlorate literature which included some human studies not available at the time of the ECAO report (PSG, 1994) At the June 1994 meeting ECAO defined the need for an extended literature review of perchlorate which specifically focuses on quantitative data regarding uncertainty factors normally used by ECAO's RfD Working Group in establishing reference doses. This report is intended to summarize these pertinent data.

## 1.3 *Perchlorate Carcinogenicity*

The USEPA Risk Assessment Forum discussed overall mechanisms of thyroid follicular cell carcinogenesis by goitrogens which block iodine uptake (USEPA, 1988; Hill et al, 1989). Experimental perchlorate thyroid carcinogenesis requires goitrogenesis and, hence, is mediated by the same iodine-blocking mechanism(s) which cause depression of T3/T4 and elevation of TSH, cardinal signs of disturbance of the thyroid-pituitary axis. If the thyroid-pituitary axis is not disturbed, there is no carcinogenic

risk. Animals treated with perchlorate at carcinogenic levels are prevented from thyroid carcinogenesis if given exogenous T3/T4 (Paynter et al, 1988). Hence, the threshold concentration of perchlorate below which there is no depression of T3/T4 with TSH elevation is completely protective for carcinogenesis. Both the Paynter et al (1988) and Hill et al (1989) papers represent USEPA's endorsement of a threshold mechanism for thyroid follicular carcinogenesis which depends upon goitrogenesis resulting from derangement of the thyroid-pituitary axis, *i.e.*, depressed T3/T4 with elevated TSH. For these reasons, despite USEPA's classification of B2 carcinogenicity, there is no carcinogenic risk from perchlorate at levels below the NOAEL for disruption of the thyroid-pituitary axis. Hence, carcinogenesis as an endpoint will not be discussed further in this paper and the discussion instead will focus on setting a reference dose (RfD) for noncarcinogenic effects.

### 1.4 *NOAEL/LOAEL Values*

Reference doses (RfDs) for the protection of human health are optimally derived from human data, if available. Because of its widespread use in the chemotherapy of hyperthyroidism, as much human data exists for perchlorate as for any other potentially toxic substance. For other less well studied toxicants, precise dosimetry is derived from animal experiments and adjusted for human risks via application of safety factors (Dourson, 1994). Critical values in the determination of safe doses are the NOAEL (No Observed Adverse Effect Level) and LOAEL (Lowest Observed Adverse Effect Level), the highest dose showing no adverse health effect and the lowest dose showing such an effect, respectively.

A careful compilation of available studies of the perchlorate toxicity database shows a consistency of effects across species and sensitive individuals with NOAEL and LOAEL values agreeing within the same order of magnitude. Hence, it is recommended that no safety factors should be used for database insufficiency or for interspecies extrapolation (see further discussion in Section 5.0).

## 2.0 *STUDIES OF PERCHLORATE TOXICITY IN HUMANS*

Most studies of perchlorate in humans and experimental animals have utilized doses ≥5 mg/kg/day; fewer contain the low dose information relevant to the establishment of a dose-response relationship between perchlorate exposure and disturbance of the thyroid-pituitary axis, which is the most sensitive measure of perchlorate toxicity. Much of the human data concerning perchlorate toxicity come from case studies of hyperthyroid patients treated with perchlorate to reduce thyroid volume. There are less data concerning NOAEL and LOAEL values in normal

humans. The one epidemiologic study of perchlorate workers could not discern the effects of perchlorate itself, since many chemical exposures existed simultaneously (Rockette and Arena, 1983).

## 2.1 *Studies in Normal Human Volunteers*

Three studies of normal human volunteers treated with perchlorate exist. They are Brabant et al (1992, 1994, 1995), Burgi et al (1974) and Shigan (1963). These studies are summarized below.

### 2.1.1. *Brabant et al (1992, 1994, 1995)*

Perchlorate continues to be utilized in Germany for the control of hyperthyroidism. Dr. Georg Brabant, a clinical endocrinologist at the Medizinische Hochschule in Hannover, has been conducting research into the mechanisms of perchlorate action for the past five years. In the Brabant et al (1992) study, five healthy male volunteers in their mid-20s were treated for four weeks with 200 µg/day iodine followed by 900 mg/day (12 mg/kg/day) perchlorate for an additional four weeks. T3/T4 and TSH levels were followed during a 24 hours period at the end of both iodine and perchlorate treatments. T3/T4 levels were not altered by perchlorate treatment and TSH levels *decreased* slightly during the four weeks on perchlorate. Hence, from the published Brabant et al (1992) study, a human NOAEL for perchlorate for perturbation of the thyroid-pituitary axis appears to be 12 mg/kg/day.

Although the above former technical definition of a NOAEL for perchlorate induction of hypothyroidism was satisfied (lack of T3/T4 depression, no elevation of TSH), Brabant et al (personal communication, 1994, 1995) in further experiments with healthy male volunteers have shown that treatment with 12 mg/kg/day perchlorate for longer than four weeks results in a slight, but statistically significant increase in thyroid volume for all treated human subjects, even though TSH is never seen to increase. Their interpretation is that enhanced thyrocyte sensitivity to TSH is an adaptive response which is as important as increased TSH levels in the human response to inhibited iodine uptake. Hence, 12 mg/kg/day is better interpreted as a human NOAEL/LOAEL, *i.e.*, at the interface between NOAEL and LOAEL values, rather than as a strict NOAEL *per se*.

Dr. Brabant is in the process of writing up his latest research for publication in a peer-reviewed journal (Brabant, 1995). Within the past year his group has realized that subtle, diurnally adjusted changes in thyroglobulin levels may be the most sensitive index of perchlorate activity. It is anticipated that this endpoint may allow precise titration of a human NOAEL. In addition, longer term followup of his volunteer cohort

after cessation of perchlorate treatment has shown that slight increases in thyroid volumes quickly return to normal.

2.1.2 *Burgi et al (1974)*

Burgi et al (1974) examined the effect of 200 mg perchlorate administered three times daily for one week to five healthy volunteers on the fate of radioiodines administered 17 days (as iodine-125) and 6 days (as iodine-131-thyroxin) previously. Average weight of these five volunteers was 61.8 kg. Burgi et al's complicated protocol was designed to determine if perchlorate could displace all incorporated radioiodines from the human thyroid gland. Since part of the endogenous radioiodine was purged from thyroid glands in this study, **9.7 mg/kg/day** is calculated as a **LOAEL** sufficient for disturbance of the thyroid-pituitary axis.

2.1.3. *Shigan (1963)*

Shigan (1963) utilized urinary excretion of administered iodine-131 as a measure of iodine uptake in normal human healthy volunteers fed ammonium perchlorate. Four of 5 volunteers had increased urinary excretion of iodine-131 following ingestion of **2.9 mg/kg/day**, which is a **LOAEL** in this study. Further details concerning this Russian study are not available, but these human data compared favorably with experimental data from rats reported in the same paper (see discussion below).

## 2.2 *Studies in Hyperthyroid Patients*

Although case studies are somewhat useful in the absence of valid epidemiologic data, they are limited in their statistical power. In all but one case exposed to perchlorate for only a few hours, treatment was prolonged at very high doses of perchlorate and determination of NOAEL or LOAEL levels is precluded. The one exception comes from the short-term study of Stanbury & Wyngaarden (1952) in which one hyperthyroid patient showed a **LOAEL of 1.4 mg/kg/day** and a **NOAEL of 0.14 mg/kg/day**.

Chronic treatment of Graves' disease patients with high levels of perchlorate has resulted in agranulocytosis and aplastic anemia. It is most likely that secondary chronic effects of perchlorate administration are mediated by loss of T3/T4 and the hormonal effects of subnormal triiodothyronine and thyroxin on bone marrow production of blood cells. Higher levels of perchlorate are required for methemoglobulinemia and irritation of oral or gastric mucosa, eyes or skin. Hence, overall, levels which are protective of thyroid-pituitary axis dysfunction, those endpoints

utilized for NOAEL/LOAEL/RfD calculations, are protective for all known adverse effects of perchlorate.

In patients treated with perchlorate at >15 mg/kg/day for Graves' disease, some skin rashes (6/240), nausea (5/240) and agranulocytosis (1/240) were seen (Crooks and Wayne, 1960). Additional cases of agranulocytosis were also described by Barzilai and Sheinfeld (1966), Southwell and Randall (1960) and Sunar (1963). In Morgans and Trotter (1960), 3% of 180 patients treated with ≥6 mg/kg/day and 18% of 67 patients treated with ≥20 mg/kg/day perchlorate developed skin rashes, sore throats and gastrointestinal irritation. One fatal acute liver atrophy developed in a patient treated with 10 mg/kg/day perchlorate for 13 months (Kotzaurek, 1965). Another case report describes nephrotic syndrome in a patient treated with 11 mg/kg/day for 5 months (Weber and Wolf, 1969). In the 1960s, several patients receiving ≥10 mg/kg/day perchlorate for Graves' disease therapy developed fatal aplastic anemias (Hobson, 1961; Johnson and Moore, 1961; Fawcett and Clarke, 1961; Krevans et al, 1962; Gjemdal, 1963; Barzilai and Sheinfeld, 1966). Although these side effects of prolonged perchlorate treatment are serious, two constraints on the interpretation for setting a reference dose must be noted:

- None of the observed side effects occurred in patients who received less than 6 mg/kg/day perchlorate.
- Similar side effects have been noted in other therapeutic regimes for hyperthyroidism, including propylthiouracil and carbimazole (Everd, 1976; Biswas et al, 1991). Use of these chemotherapeutic regimes also required high doses for elicitation of adverse effects.

## 3.0 *STUDIES OF PERCHLORATE TOXICITY IN ANIMALS*

In general, perchlorate toxicities in domestic or experimental animals mirror those seen in human volunteers or patients receiving perchlorate therapeutically. Disruption of the thyroid-pituitary axis is the main mammalian toxicity of perchlorate and a threshold for this effect is seen in animals as well as in humans. As a beneficial effector, perchlorate salts have been utilized in Russia for increasing the weight of domestic animals (Yakimenko et al, 1981). Weight gains of up to 31 percent as compared to controls were seen with 5 mg/kg/day ammonium perchlorate, comparable to the LOAEL values seen in other studies.

### 3.1 *Acute Studies in Experimental Animals*

There are many reported acute toxicity experiments of perchlorate administration to animals. The LD50 in rats is in the range of 1-4 g/kg, *i.e.*, perchlorate is moderately toxic (Praeger and Sax, 1982). However, acute

toxicity experiments are of little value in establishing a reference dose and, therefore, are not considered further here.

### 3.2 *Chronic Studies in Experimental Animals*

The only chronic studies of perchlorate toxicity in experimental animals have to do with carcinogenesis and, as discussed above, are not directly relevant to establishment of a reference dose. Studies are summarized below which clearly demonstrate perchlorate not to be an initiator of thyroid carcinogenesis.

Hiasa et al (1987) fed male Wistar rats 0 or 1000 ppm perchlorate for 20 weeks and measured T3, T4 and TSH levels, as well as body and liver weights and appearance of thyroid tumors. Although TSH levels increased, T3 and T4 changes were not statistically significant. No thyroid tumors were seen. In a separate groups of 20 rats first injected with 28 mg/kg N-bis(2-hydroxypropyl)nitrosamine and then fed perchlorate, 20/20 developed thyroid tumors whereas without perchlorate treatment only 1/20 developed tumors. There were no effects of perchlorate on body or liver weights. Exactly the same promotion of initiated thyroid tumors may be mediated by iodine deficient diets alone, suggesting that this is the sole activity of perchlorate (Ohshima and Ward, 1986).

The experimental studies of perchlorate dose-response for perturbation of the thyroid-pituitary axis and mechanism of carcinogenesis are prototypic in demonstrating a threshold for carcinogenic promotion. The evidence for thyroid tumor promoter thresholds has been summarized by USEPA (USEPA, 1988; Paynter et al, 1988; Hill et al, 1989) and the implications for risk assessment of perchlorate and other nongenotoxic thyroid tumor promoters reviewed recently by McClain (1992).

### 3.3 *Subchronic Studies in Experimental Animals*

Whereas most laboratory studies of perchlorate carcinogenicity using experimental rodents have been chronic, most dose-response studies have been subchronic. Of particular value is Männistö et al (1979), a four day study in rats which received four concentrations of perchlorate in drinking water, which concentrations embraced NOAEL and LOAEL doses for perturbation of the thyroid-pituitary axis.

The data of Männistö et al (1979) are useful in that a wide range of perchlorate doses were administered. These data are summarized in the following table:

Table 1
T3/T4 and TSH Levels in Perchlorate-Treated Rats (Männistö et al, 1979)

| Perchlorate (mg/L) | mg/kg/day | T3/T4 | TSH |
|---|---|---|---|
| 0 | 0 | No change | No change |
| 10 | 1.5 | No change | No change |
| 50 | 7.6 | Decrease | Sl. increase* |
| 100 | 15.3 | Decrease | Increase |
| 500 | 76.3 | Decrease | Increase |

*The slight increase in TSH was not statistically significant.

Given the definition of perturbation of the thyroid-pituitary axis, that T3/T4 levels must be depressed while TSH is elevated, both in statistically significant manners, from the Männistö et al (1979) data it is concluded that 7.6 mg/kg/day is a NOAEL and 15.3 mg/kg/day is a LOAEL for perchlorate in the rat.

Comparison of the Männistö et al (1979) and Brabant et al (1992) LOAEL data challenges the assumption that rats have a different sensitivity to perchlorate than humans. Whereas rats showed a slight, statistically significant, increase in TSH at 15.3 mg/kg/day perchlorate, TSH remained depressed at 12 mg/kg/day in human volunteers, although (Brabant et al, 1994) thyroid volume increased slightly after four weeks. Hence, the LOAELs derived from these studies are similar: 15.3 and 12 mg/kg/day, in the rat and in humans, respectively.

Kessler and Kruskemper (1966) fed 1 percent potassium perchlorate (~1300 mg/kg/day) to 40 rats maintained with 40 controls. Groups of 6-8 rats were sacrificed immediately and after 40, 120, 220 and 730 days of treatment. There was no influence of 1300 mg/kg/day on body weight. Thyroid glands, however, were hypertrophied, with histological changes being detected by 40 days and progressing throughout the experiment through fibrosis and on to follicular adenomas. Gauss (1972) appears to have conducted similar experiments in mice, with similar effects seen on the thyroid gland, but also minor weight loss (11.6 percent) during the first two months of treatment (as cited in ECAO, 1992).

To study iodine uptake by the rat and rabbit thyroid Shigan (1963), test doses of iodine-131 were given a day following ingestion of perchlorate at

doses of 0, 0.25, 2 and 40 mg/kg/day. Urinary excretion of iodine-131 was higher than controls in the 2 and 40 mg/kg/day groups, although not higher at 40 than 2 mg/kg/day. By this criterion, 0.25 mg/kg/day perchlorate did not block iodine-131 uptake and was the NOAEL in this study, while 2.0 mg/kg/day was the LOAEL.

### 3.4 *Other Organ Site Studies in Experimental Animals*

Pflugfelder (1959) studied the effect of ingested potassium perchlorate on the thyroid and other organs of the chicken. Daily doses were 20, 30 and 40 mg/kg to groups of three chickens for each dose. Although it appears as if this was a chronic study, it is not clear how long chickens were maintained on these perchlorate doses prior to sacrifice and necropsy. Thyroid volumes were reduced at all doses as was body weight gain. Other organ toxicities noted at all doses included: Failure of the bursa of Fabricius (an organ for the maturation of T-lymphocytes), diminished feather exfoliation, lessened sexual development and degeneration of cerebellar Purkinje cells. Hence, in the chicken, it appears that 20 mg/kg/day perchlorate is a LOAEL for several target organ sites, albeit with limited significance for assessing human risks.

Sreebny et al (1963) studied the effects of drinking water with 1 percent $KClO_4$ to male Sprague-Dawley rats for 30 or 60 days on three exocrine glands: The submaxillary gland, parotid gland and pancreas. Although submaxillary gland and pancreatic weights were reduced as was amylolytic activity of the parotid, these effects were also caused by propylthiouracil and followed induction of thyroid hyperplasia in both cases. Hence, the observed effects on exocrine glands in this experiment were indirectly mediated by activity of perchlorate on the thyroid gland.

Hiasa et al (1987) found no effects on liver or body weights from feeding 1000 ppm perchlorate to male rats for 20 weeks.

Shigan (1963) studied effects of perchlorate in rats and rabbits dosed with 0.25, 2 and 40 mg/kg/day orally. Several measured endpoints were negative at all doses:

- Involuntary regulation of cardiac activity,
- Central nervous system functions,
- Hemoglobin synthesis,
- Protein synthesis, and
- Liver function.

Hence, for organs other than the thyroid, the NOAEL for perchlorate in rats and rabbits is in excess of 40 mg/kg/day.

## 3.5 *Reproductive, Developmental and Genotoxicity Studies*

### 3.4.1. *Reproductive and developmental toxicity studies*

Postel (1957) fed pregnant guinea pigs with 1 percent potassium perchlorate (740 mg/kg/day) in drinking water from the 21st through the 48th day of gestation and noted a 15-fold enlargement of fetal thyroids. This fetotoxic dose is 100 times the NOAEL seen in other experimental studies. The same treatment regimen was too short to enlarge adult guinea pig thyroids, which required 60 or more days of exposure to perchlorate for thyroid enlargement.

Brown-Grant (1966) also fed potassium perchlorate at 1 percent in drinking water (740 mg/kg/day) to gravid Wistar rats from the 2d to the 8th day of gestation. One percent KCl served as the control dose. Live litter births occurred in 8/11 perchlorate-treated and 7/11 KCl-treated groups. The dams which had not given birth in both groups showed no signs of implantation.

In a subsequent study, Brown-Grant and Sherwood (1971) also fed potassium perchlorate at 1 percent in drinking water to gravid Wistar rats, but in this case the rats were lactating, to delay implantation, and 0.1 percent KI served as the control. The feeding scheme was also prolonged from the day of conception to the 12th or 13th day. Again, there was not a significant effect of perchlorate on implantation. When pup thyroids were examined, a significant increase in weights of the perchlorate-treated group was noted (~50 percent), which is similar to the adult response at this high dose. Although perchlorate can cross the placenta, it does not affect blastocyst survival in the rat. It is concluded that at 100 times the NOAEL, perchlorate crosses the placenta where its only effect is to enlarge the fetal thyroid gland. Perchlorate is not a reproductive or developmental toxin at this very high dose.

### 3.4.2. *Genotoxicity data*

A thorough review of all available scientific literature reveals no tests for genotoxicity of perchlorate. Chemically, there is no reason to suspect that perchlorate would react with DNA or that its presence in cells would prove disruptive to chromosomal structure or replication. The mechanism by which perchlorate is carcinogenic for the thyroid gland at high concentrations requires blocked iodine uptake and subsequent goitrogenesis and does not depend upon any genotoxic activity. Given

the known mechanism of goitrogenesis and lack of data for mutagenicity or clastogenicity, it is concluded that perchlorate is not genotoxic.

## 4.0 *TABULAR COMPILATION OF THOSE STUDIES WHICH ARE MOST RELEVANT FOR ESTABLISHING A REFERENCE DOSE*

A plethora of data exists for high doses of perchlorate administered to hyperthyroid patients. Although relatively few normal human and experimental studies of perchlorate toxicity are available from which to evaluate dosimetry, the agreement between all these various studies is striking. These data are best compared tabularly.

### 4.1 *Human NOAEL and LOAEL Values*

Three studies have been conducted in normal human volunteers administered perchlorate for various periods of time. In the first Brabant study (Brabant, 1992), as measured by T3/T4 dimunition and TSH stimulation, there were no effects in five healthy volunteers given 12 mg/kg/day after four weeks. However, in a followup study utilizing another five healthy volunteers, although the T3/T4/TSH levels were not perturbed, some increase in thyroid volume occurred after administration of 12 mg/kg/day beyond the fourth week. These data along with those of Burgi et al (1974) and Shigan (1963 are summarized in the following table.

Table 2
**Comparison of NOAEL and LOAEL Values in Human Studies**

| Reference | NOAEL | LOAEL | Effect |
|---|---|---|---|
| Brabant et al (1992) | 12 mg/kg/d | | T3/T4 decrease<br>TSH increase |
| Brabant et al (1994) | | 12 mg/kg/d | Thyroid volume increase |
| Burgi et al (1974) | | 9.7 mg/kg/d | Depletion of I-131 from thyroid |
| Shigan (1963) | | 2.9 mg/kg/d | Depletion of I-131 from thyroid |

It may be seen that these values are in fair agreement over a four-fold range and, as will be summarized below, also in fair agreement with data obtained from hyperthyroid patients and in experimental animals.

All volunteers in both Brabant et al (1992, 1994) studies and the Shigan (1963) study were male. However, in the Burgi et al (1974) study, of the five volunteers, two were male and three female. Hence, it is possible from their experimental data to assess the overall sensitivities of human male vs. female thyroids to 200 mg perchlorate being administered three times daily for a week. These data are especially important given the paucity of experimental data in female test animals.

Data from the Burgi et al (1974) study are segregated for normal male and female human subjects and presented in Table 3. Doses of perchlorate, when corrected for body weights, were less in males (8.2 mg/kg/day) than females (11.1 mg/kg/day). No difference was seen between males and females in their urinary excretion of radiolabeled thyroid iodine after treatment with 600 mg perchlorate daily for a week. Males show a 236 percent increase in urinary radioiodine whereas females show a 215 percent increase as measures of mobilization of thyroid iodine by perchlorate. These values are statistically indistinguishable.

Table 3
Comparison of Perchlorate Toxicities Between Males and Females
(Data taken from Burgi et al, 1974)

| | | Thyroid Effects** | | |
|---|---|---|---|---|
| Sex (No.) | Dose* | (1) | (2) | (3) |
| Male (2) | 8.2 | 93 | 220 | 236 |
| Female (3) | 11.1 | 101 | 217 | 215 |

*Doses in mg/kg/day for average weight of males at 73 kg and females at 54 kg.
**The following thyroid effects are corrected for relative body weights:
(1) Urinary excretion of radioiodine (µg/day) before perchlorate.
(2) Urinary excretion of radioiodine (µg/day) after perchlorate.
(3) Percent increase in urinary radioiodine after perchlorate treatment.

It is concluded that normal males and females have identical sensitivities to the action of perchlorate on the thyroid gland.

Table 4 (next page) compiles LOAEL data from 11 studies of hyperthyroid patients for whom perchlorate was used therapeutically.

Table 4
**LOAEL Values in Hyperthyroid Patients**

| Reference | LOAEL (mg/kg/d) |
|---|---|
| Stanbury & Wyngaarden (1952) | 1.4 |
| Crooks & Wayne (1960) | 15 |
| Morgans & Trotter (1960) | 6 |
| Kotzaurek (1965) | 10 |
| Weber & Wolf (1969) | 11 |
| Hobson (1961 | |
| Johnson & Moore (1961) | |
| Fawcett & Clarke (1961) | All |
| Krevans et al (1962) | ≥10 |
| Gjemdal (1963) | |
| Barzilai & Sheinfeld (1966) | |

It may be seen that there is fair agreement between LOAEL values as determined from case studies and the data discussed previously from normal healthy volunteers.

4.2 - *Experimental Animal NOAEL and LOAEL Values*

The results of previously discussed experimental studies are also presented below in tabular form to facilitate comparison of multiple studies on perchlorate toxicity. Following a summary of NOAEL and LOAEL values in experimental animals, these are compared with human values in order to gain an overall appreciation of the general agreement of data across species.

Table 5 (next page) summarizes NOAEL and LOAEL values for perchlorate in three animal species for the thyroid and other organ sites.

415 744 2180

Table 5
Interspecies Comparison of NOAEL and LOAEL Values

| Reference | mg/kg/day NOAEL | LOAEL | Species | Target Organ |
|---|---|---|---|---|
| Männistö et al (1979) | 7.6 | 15.3 | rat | thyroid |
| Shigan (1963) | 0.25 | 2.0 | rat rabbit | thyroid |
| Shigan (1963) | 40 | | rat rabbit | other organs |
| Pflugfelder (1959) | | 2.0 | chicken | other organs |

As mentioned earlier, virtually all animal studies have employed male test animals, usually male rats, and there are no animal data from which to adjudge male vs. female comparisons. However, some reproductive studies have been done for perchlorate administration to pregnant dams and these results are summarized below.

Table 6
Summary of Reproductive Data in Animal Studies

| Reference | Dose | Reproductive Effect |
|---|---|---|
| Postel (1957) | 740 mg/kg/d | Fetal thyroid enlargement |
| Brown-Grant (1966) | 740 mg/kg/d | None |
| Brown-Grant and Sherwood (1971) | 740 mg/kg/d | Fetal thyroid enlargement |

Other than the known thyrotoxic effects, there are no reproductive effects of perchlorate administration at very high doses to gravid test animals.

In addition to not being a reproductive toxin, there also are no data to indicate that perchlorate is genotoxic.

The following table summarizes data for target organ sites other than the thyroid gland. Since some organ toxicities depend upon the known influence of perchlorate on the thyroid and, hence, represent indirect effects, this column is included in the table to facilitate interpretation.

**Table 7**
**Summary of Target Organ Data in Animal Studies**

| Target Organ | Toxicity | Mechanism of action |
|---|---|---|
| Thyroid gland | + | Blocked iodine transport |
| Liver | - | n/a |
| Heart | - | n/a |
| Central nervous system | - | n/a |
| Bone marrow | + | Thyroxin-dependent* |
| Submaxillary gland | + | Thyroxin-dependent* |
| Parotid gland | + | Thyroxin-dependent* |
| Pancreas | + | Thyroxin-dependent* |

*Toxicities which are mediated through impaired T3/T4 synthesis by the thyroid gland.

Summarizing the three previous discussions, males and females have identical sensitivities to perchlorate toxicity, perchlorate is neither a reproductive nor genotoxic substance and the only additional organ sites adversely affected by perchlorate administration besides the thyroid gland itself are those organs whose homeostasis depends upon thyroxin. As indicated in the discussions above, overall there is a surprising concordance of data concerning NOAEL and LOAEL values for the effects of perchlorate on the thyroid gland in normal human subjects, hyperthyroid patients and experimental animals. These data are summarized in total in Table 8 (next page).

Table 8
**Comparison of Human and Animal NOAEL and LOAEL Values for Perchlorate Toxicity to the Thyroid Gland**

| Reference | NOAEL (mg/kg/d) | LOAEL (mg/kg/d) |
|---|---|---|
| *Studies in Normal Human Volunteers* | | |
| Brabant et al (1992) | 12 | |
| Brabant et al (1994) | | 12 |
| Burgi et al (1974) | | 9.7 |
| Shigan (1963) | | 2.9 |
| *Studies in Hyperthyroid Patients* | | |
| Stanbury & Wyngaarden (1952) | 0.14 | 1.4* |
| 13 Subchronic Studies | | 6.0 |
| *Studies in Experimental Animals* | | |
| Männistö et al (1979) | 7.6 | 15.3 |
| Shigan (1963) | 0.25 | 2.0 |
| Pflugfelder (1959) | | 20** |

*An acute study with endpoints measured within hours.
**This was the lowest dose tested for thyrotoxicity in chickens.

It should be noted that all subchronic NOAEL and LOAEL values summarized above in normal humans, hyperthyroid patients and test animals are within an order of magnitude (2-20 mg/kg/day), except for the 0.25 mg/kg/day NOAEL of Shigan (1963). However, the Shigan (1963) doses studied dropped from 2.0 to 0.25 mg/kg/day, with no doses in between. Obviously even lower NOAELs could have been found in this study if, for example, the next lower dose after 2.0 mg/kg/day had been 0.1 or 0.05 mg/kg/day.

## 5.0 DISCUSSION OF SAFETY FACTORS

In the subsections below, the relevance of the above-summarized data to establishing safety factors for the conversion of NOAEL and LOAEL data to an oral human reference dose for perchlorate is discussed.

### 5.1 *Lack of Chronic Data and Database Sufficiency*

The available studies in human volunteers have all been terminated after a relatively brief exposure period (1-6 weeks). Brabant et al (1994, 1995) noted that it took longer than four weeks of exposure for his healthy volunteers to develop slightly increased thyroid volume, even though other cardinal signs of hyperthyroidism (T3/T4 dimunition, TSH increase) had not occurred. Hence, it would appear that the NOAEL of 12 mg/kg/day perchlorate noted in the Brabant (1992) study is more precisely a NOAEL/LOAEL and similar to the LOAEL observed by Stanbury and Wyngaarden (1952) of 1.4 mg/kg/day, albeit from an acute study in one hyperthyroid patient.

The lowest LOAEL of 6 mg/kg/day discussed in this report comes from the brief review of Morgans and Trotter (1960) in which 6/180 hyperthyroid patients developed skin rashes, sore throats and gastrointestinal irritation after a few weeks treatment with perchlorate. These epithelial reactions in only 3 percent of treated patients may reflect immunoglobulin-mediated hypersensitivity to perchlorate in this small minority. The dose range of these patients was actually 400-1000 mg/day and body weights of the six patients who developed symptoms is not given. If body weights of the afflicted individuals were 50 kg rather than the 70 kg assumed for calculation of the 6 mg/kg/day LOAEL and if they had been exposed to 1000 mg/day rather than the 400 mg/day assumed, then this LOAEL might be more in the range of other values seen (≥10 mg/kg/day). Unfortunately, the raw data required for these calculations are not available.

Many of the other observed toxic effects of perchlorate seen after chronic administration of ≥10 mg/kg/day, such as agranulocytosis and aplastic anemia, are mediated by impairment of bone marrow which in turn is dependent upon thyroxin for normal hematopoietic development (Wartofsky, 1994). Given the consistency of NOAEL and LOAEL values in normal human volunteers and hyperthyroid patients and similar values obtained in experimental animals, it is suggested that the NOAEL/LOAEL of 12 mg/kg/day from Brabant et al (1992, 1994, 1995) be utilized with three-fold adjustments for extrapolation from subchronic-to-chronic exposure times and potential sensitives in the human population. This brings the recommended RfD to 1.2 mg/kg/day, which

is very similar to the 1.4 mg/kg/day LOAEL observed by Stanbury and Wyngaarden (1952) in their acute experiment on one hyperthyroid patient. Given the breadth of human and experimental animal data and its consistency, it is recommended that application of no additional safety factors for database insufficiency or reproductive/developmental effects are required.

### 5.2 *Male vs. Female Data*

In the Burgi et al (1974) study of the effect of perchlorate on iodine secretion in five healthy volunteers, two were male and three were female. No differences were noted between male and female volunteers In the Brabant (1992, 1994, 1995) studies, all subjects were healthy males. In terms of perchlorate toxicities during therapy for hyperthyroidism, there have been no recorded differences between male and female patients.

It has been reported that hyperthyroidism (goiter) in humans is three times more prevalent in females than in males (Wartofsky, 1994). If this predilection for the disease were indicative of a greater sensitivity among human females to goitrogenic substances, including perchlorate, then this enhanced sensitivity would have to be taken into account while adjusting available NOAEL and LOAEL data for a safe human reference dose.

However, it appears that the various hyperthyroidisms, viz., Graves' disease, which appear exacerbated in females are the result of sensitized female autoimmunity to TSH and other receptor proteins (Wartofsky, 1994). Females in general are many times more susceptible to all autoimmune diseases such as systemic lupus erythematosus and myasthenia gravis due to their more finely tuned T-lymphocyte system which must prevent potential fetal rejection during pregnancy (Hahn, 1994).

It is recommended that no safety factors are required for ensuring that females may be more sensitive than males to perchlorate toxicity.

### 5.3 *Other Potential Sensitive Populations and Target Organs*

The population of hyperthyroid patients may be more sensitive to the actions of perchlorate than normal healthy individuals. However, rather than being adversely affected by perchlorate, it is beneficial to those afflicted by hyperthyroidism. No other more sensitive subpopulations, including fetuses, have been shown to exist. Although perchlorate passes the placenta and fetal thyroids enlarge upon daily exposure to perchlorate, this response is to levels comparable to those required for adult thyroid

enlargement. Hence, it is recommended that only a three-fold safety factor be applied for protection of potentially sensitive populations.

Clearly, perchlorate targets the thyroid gland by virtue of its competitive ability to inhibit iodine transport. All known toxicities of perchlorate to other target organs such as the exocrine and hematopoietic systems are probably mediated by thyrotoxicity and subsequent thyroxin depletion. In some described hypersensitivities in Graves' disease patients, these may include the effects of thyroxin depletion upon the immune system, especially inhibition of suppressor T-cells. The only non-thyroid-mediated toxicities of perchlorate require very high doses, in excess of grams/day, and are valueless in determination of an oral reference dose.

## 6.0 *CONCLUSIONS*

As evidenced in several previous reviews (ECAO, 1992; ICF, 1993; PSG, 1994) and herein, the database for perchlorate toxicity is replete with NOAEL and LOAEL values from normal human volunteers, hyperthyroid patients and experimental animals. The best dose-response data available from five sources of information are remarkably consistent:

- Normal human volunteers do not demonstrate any thyrotoxicity to 12 mg/kg/day perchlorate for four weeks of administration daily and only after the fifth week show a slight increase in thyroid volume (Brabant et al. 1992, 1994, 1995).
- Burgi et al (1974) determined that 9.7 mg/kg/day was a LOAEL in healthy humans for purging radioiodine from the thyroid.
- The lowest LOAEL and highest NOAEL in a hyperthyroid patient treated with perchlorate were 1.4 and 0.14 mg/kg/day, respectively (Stanbury and Wyngaarden, 1952).
- Rats treated subchronically with a range of perchlorate doses show a NOAEL at 7.6 mg/kg/day and a LOAEL at 15.3 mg/kg/day (Männistö et al, 1979).
- The LOAEL for blocking radioiodine uptake in rat and rabbit thyroids is 2.0 mg/kg/day (Shigan, 1963).

Males and females appear to have equal sensitivities to perchlorate and no other sensitive populations, except hyperthyroid cases who would be benefitted by perchlorate exposure, have been identified. All additional organ site sensitivities may be attributed to indirect effects of perchlorate on inhibition of thyroxin synthesis by the affected thyroid gland target organ site. Perchlorate is neither a reproductive toxin nor is it genotoxic.

It is recommended that, given the amount of concordant data available, the Brabant et al NOAEL/LOAEL value of 12 mg/kg/day determined in normal humans be adopted as the basis for a reference dose for application to human risk assessment. Given the plethora of human data available and its consistency with data derived from test animals along with the well understood mechanism of perchlorate toxicity, it is recommended that no safety factors for database insufficiency or developmental/reproductive toxicity be applied. Application of a three-fold safety factor for subchronic-to-chronic exposure extrapolation and an additional three-fold safety factor to account for potential sensitive human subpopulations yields a recommended oral reference dose of 1.2 mg/kg/day.

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